Anti-Inflammatory Activity of the Ethanolic Extract of *Acrostichum aureum* (Linn.) root

Hemayet Hossain[1*], Ismet Ara Jahan[1], Ishrat Nimmi[1], Md. Akbar Hossain[2] and Md Hassan Kawsar[2]

[1]BCSIR Laboratories, Bangladesh Council of Scientific and Industrial Research,
Dr. Qudrat-E-Khuda Road, Dhaka-1205, Bangladesh
[2]Department of Pharmacy, Dhaka International University, Dhaka-1213, Bangladesh

Abstract

The ethanolic crude extract of the root of *Acrostichum aureum* was evaluated for its anti-inflammatory activity. At the dose of 400 mg/kg body weight, the extract showed a significant anti-inflammatory activity in the carrageenan-induced oedema test in rats showing 65.90% reduction in the paw volume ($P<0.01$) comparable to that produced by the standard drug indomethacin (66.66%) after 24h. The obtained result tends to suggest the probable anti-inflammatory activity of the ethanolic crude extract of the root of *Acrostichum aureum* and justify its use in folkloric remedies.

Key words: *Acrostichum aureum*, Anti-inflammatory, carrageenan-induced oedema, indomethacin.

Introduction

Acrostichum aureum (*A. aureum*) Linn. (Family: Ptridiaceae) locally known as 'Tiger fern' is an evergreen shrub distributed widely throughout Bangladesh, India, USA, Brazil, China, Taiwan, Japan, Australia and Sri Lanka mostly on mangrove forests and sea coast area. The ethanolic extract of the plant contains 2-butanone, ponasterone, pterosterone, kaempferol and quercetin (Mei *et al.*, 2006). Traditionally, the roots are used to treat rheumatism, wounds and boils. Leaves are used to stop bleeding. The plant contains glycosides, saponins, steroids and fronds are used as pain-killers and stomach troubles (Burkill, 1985). The methanolic extract from *Acrostichum aureum* leaves showed selective cytotoxicity (IC_{50}: 1.02 mg/ml) against different cancer cell lines (Shaikh *et al.*, 2009).

The anti-inflammatory drugs have not been used successfully in all cases due to adverse side effects such as gastric lesions caused by non-steroidal anti-inflammatory drugs (NSAIDs). Therefore, new drugs lacking these side effects are searched for all over the world. During this process, the investigation of the efficacy of plant-based drugs used in the traditional medicines for the treatment of pain, fever and inflammatory ailments have received attention because they are cheap and have little side effects (Kumara, 2001). The present study was designed to provide scientific evidence of the claimed ethnopharmacological properties by investigating the anti-inflammatory activity of the ethanolic extract of *Acrostichum aureum* root.

Materials and Methods

Collection and identification of plant material: The plant *Acrostichum aureum* (Linn.) was collected from Karamjal area of Sundarban forest, Bangladesh in January, 2008 and was identified by Bangladesh National Herbarium, Mirpur, Dhaka (Accession number-29791).

Preparation of ethanolic extract: The collected plant parts (roots) were separated from undesirable materials and then were washed with water and air-dried under shed temperature followed by drying in an electric oven at 40ºC. The dried roots were ground into powder with the help of a suitable grinder (Capacitor start motor, Wuhu motor factory, China). The powder was stored in an airtight container and kept in a cool, dark and dry place. About 600 g of powered material was taken in a clean, flat-bottomed glass container and soaked in 2.0 L of 80% ethanol. The container with its contents was sealed and kept for a period of 6 days accompanying occasional shaking and stirring. The whole mixture then underwent a coarse filtration by a piece of clean, white cotton material. Then it was filtered through whatman filter paper (Bibby RE200, Sterilin Ltd., UK) and the filtrate was concentrated with rotary evaporator at a bath temperature

* Correspondence to: Md. Hemayet Hossain, Chemical Research Division, BCSIR Laboratories, Dhaka, Bangladesh Council of Scientific and Industrial Research (BCSIR), Dhaka-1205, Bangladesh. E-mail: hemayethossain02@yahoo.com

not exceeding 40˚ to have gummy concentrate extract (yield approx. 5.9%).

Test for different chemical groups: The crude ethanolic extract was tested for its different chemical groups as alkaloids, flavonoids, gums, reducing sugars, saponins, steroids and tannins (Evans, 1989). In each test 10% (w/v) solution of the ethanol extract was taken.

Test for anti-inflammatory activity

Test Animals & Drugs: Male albino rats of Wistar strain weighing between 175-202 g were used for *in vivo* anti-inflammatory screening. They were housed in standard environmental conditions at animal house of Chittagong Laboratories, BCSIR, Chittagong and fed on Balanced Trusty Chunks and water ad libitum. The cages were cleaned once daily. This study was carried out following approval from the ethical committee on the use and care of animals of the BCSIR. Indomethacin (Square Pharmaceuticals Ltd, Bangladesh) was used as standard drug for this study.

Carrageenan-induced oedema test: The method of Lanhers (Lanhers *et al.*, 1991) was adopted for the carrageenan-induced oedema test in rats. Briefly, oedema was induced by injecting 0.05 ml of 1% carrageenan into the sub plantar region of the right hind paw of each rat. Four groups (five animals per group) were used in this study. The extract was administered orally at 200 and 400 mg/kg body weight of the test groups, the negative control group received 0.5 ml of distilled water and the positive control group received 10 mg/kg body weight of indomethacin orally, 30 min before the carrageenan injection. The paw volume was measured with a micrometer screw gause at 0, 0.5, 1, 2, 4, 6 and 24 h after the administration of the drug and the extract. The percentage inhibition of inflammatory effect of the extract was calculated using the following expression:

$$\% \text{ inhibition of inflammation} = [(V_c - V_t)/V_c] \times 100$$

Where V_c is the average degree of inflammation by the control group and V_t is the average degree of inflammation by the test group.

Results and Discussion

Chemical group test: Results of different chemical tests on the ethanolic extract of *A. aureum* showed the significant presence of flavonoids and presence of tannins,reducing sugars, gums and saponins (Table 1).

Table 1. Results of different group tests of ethanolic extract of *A. aureum* root.

Alkaloid	Reducing Sugar	Tannin	Gum	Flavonoid	Saponin	Steroid
-	+	+	+	+++	+	-

+: Positive result; - : Negative result; +++: significantly positive

Anti-inflammatory activity: Table 2 showed the anti-inflammation effect of the ethanolic extract of *A. aureum* using carrageenan-induced oedema tests. In the carrageenan-induced oedema test, a maximum oedema paw volume of 1.72 ± 0.09 mm was observed in the control rats, 6 h after the carrageenan injection. The percentage inhibition of the oedema paw volume by the 400 mg/kg body weight of the extract was statistically significant compared favorably with the indomethacin treated animals at 1, 2, 4, 6 and 24 h. The maximum reduction in the paw volume by the 400 mg/kg body weight was 65.90% compared to the indomethacin (66.66%) at 24 h.

Acute inflammation involves the synthesis or release of mediator at the injured site. These mediators include prostaglandins, especially the E series, histamine, bradykinins, leucotrienes and serotonin, all of which also cause pain and fever (Silbernagel and Lang, 2000). Therefore, inhibitions of these mediators from reaching the injured site or from bringing about their pharmacological effect will normally ameliorate inflammation, pain and fever (Sawadogo *et al.*, 2006).

Carrageenan-induced paw oedema as an *in vivo* model of inflammation has been frequently used to asses the anti-edematous effect of natural products (Mani *et al.*, 2008). It has also been reported that various mediators are released by carrageenan in the rat paw while the initial phase may be due to the release of histamine, the second phase attributed to the release of prostaglandins (Mossai *et al.*, 1995). Development of oedema induced by carrageenan is commonly correlated with the early exudative stage of inflammation, one of the important processes of

inflammatory pathology (Ozaki, 1990). The 400 mg/kg dose of the ethanolic extract of *A. aureum* was the most potent and produced anti-inflammatory effect (65.90%) which was similar to indomethacin (66.66%), a well known prostaglandin inhibitor. Therefore, the anti-inflammatory property of this extract could be due to its ability to inhibit the cyclooxygenase pathway (Garcialeme *et al*., 1973).

Table 2. Effect of ethanol extract of *A. aureum* root and indomethacin on carrageenan-induced oedema paw volume in male Wistar rats.

Treatment groups	Doses (mg/kg body weight)	Right Hind Paw Volume (mm)					
		0.5h	1h	2h	4h	6h	24h
Control	0	1.01 ± 0.09*	1.30 ± 0.06*	1.49 ± 0.08*	1.69 ± 0.05*	1.72 ± 0.09*	1.32 ± 0.07*
Positive Control (Indomethacin)	10	0.49 ± 0.06* (51.48)	0.62 ± 0.07** (52.30)	0.70 ± 0.05* (53.02)	0.59 ± 0.07** (65.08)	0.58 ± 0.04** (66.27)	0.44 ± 0.09* (66.66)
Extract	200	0.99 ± 0.08* (1.98)	1.08 ± 0.06* (16.92)	1.14 ± 0.05* (23.48)	1.23 ± 0.07* (27.22)	1.17 ± 0.04* (31.97)	0.81 ± 0.09* (38.64)
Extract	400	0.54 ± 0.04* (46.53)	0.65 ± 0.03* (50.00)	0.74 ± 0.09** (50.33)	0.62 ± 0.07** (63.31)	0.59 ± 0.08* (65.69)	0.45 ± 0.06** (65.90)

Values in brackets denote percentage inhibition of the oedema paw volume.
Values are expressed as mean±SD; $^{*}P < 0.05$; $^{**}P < 0.01$ vs. control; n = 5.

In conclusion, it can be concluded that the ethanolic crude extract of *Acrostichum aureum* root possesses anti-inflammatory activity. However, further researches are necessary to find out the active principles responsible for this activity.

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